

Continuous Reproduction in the Protobranch Bivalve *Solemya reidi* (Cryptodonta: Solemyidae)¹

by

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Abstract. Specimens of *Solemya reidi* Bernard, 1980, were obtained at regular intervals from a population in Alberni Inlet, Vancouver Island, Canada, between August 1982 and October 1983. Mature individuals of both sexes (≥ 25 mm in shell length) were examined for their degree of reproductive development based on gonad histology. Female reproductive condition was also analyzed on the basis of oocyte size frequencies. Several lines of evidence indicated that spawning occurred throughout the year within this population. A synchronous bimodality in individual oocyte size frequencies persisted during the study period and a resting phase was not apparent in female gonads. Oocyte size-frequency distributions revealed that oocyte development was asynchronous within the population. Mature spermatozoa were present in males in all seasons except for the occasional partially spent individual. In addition, spawning of laboratory held specimens occurred in all seasons of the year. We conclude that *S. reidi* reproduces year round in Alberni Inlet, with a proportion of the population capable of spawning at any time of year.

INTRODUCTION

Solemya reidi Bernard, 1980, is a gutless protobranch bivalve found from southern California to the Alaskan panhandle, at depths ranging from 40 to 600 m (BERNARD, 1980), in marine habitats where oxygen and reduced sulfur compounds are simultaneously available, such as near sewage outfalls (FELBECK, 1983; FELBECK *et al.*, 1983) and beneath log-booming grounds in the Pacific Northwest (REID, 1980; REID & BERNARD, 1980). Chemoautotrophic bacterial symbionts, found in certain cells (bacteriocytes) of the large gills of all solemyids thus far examined, are proposed to provide nutrition to the clams via synthesis of reduced carbon and nitrogen compounds (FELBECK, 1983; FELBECK *et al.*, 1983).

As a consequence of its relatively easy accessibility and maintenance, *Solemya reidi* is increasingly being used as a representative species in studies of the association between chemoautotrophic endosymbionts and host animals from sulfide-rich habitats (FELBECK, 1983; FELBECK *et al.*, 1983;

HAND & SOMERO, 1983; FISHER & CHILDRESS, 1984; McMAHON & REID, 1984; POWELL & SOMERO, 1985, 1986). However, its reproduction has remained largely unexplored. The present study was designed to provide a description of the reproductive cycle in *S. reidi* in its natural habitat.

Numerous methods have been employed to measure the reproductive condition of bivalve gonads (see SASTRY, 1979 for a partial review). The vast majority of studies have relied on the subjective grading of histological sections of gonads to produce a "maturity index" (GRANT & TYLER, 1983a). A more objective utilization of female gonad sections in bivalves is to measure the oocytes and group them into size classes for analysis (SASTRY, 1979; GRANT & TYLER, 1983b). In this study, the reproductive cycle of *Solemya reidi* was investigated through analysis of gonad histology and oocyte size frequencies.

MATERIALS AND METHODS

Specimen Collection

Adult specimens of *Solemya reidi* were collected from August 1982 to October 1983 from a depth of 40 m with a Van Veen grab in the vicinity of log-booming grounds number 27 and 29 in Alberni Inlet on the west coast of Vancouver Island, B.C., Canada (49°12'N, 124°49'W). Specimens were processed for histology within 24 h of

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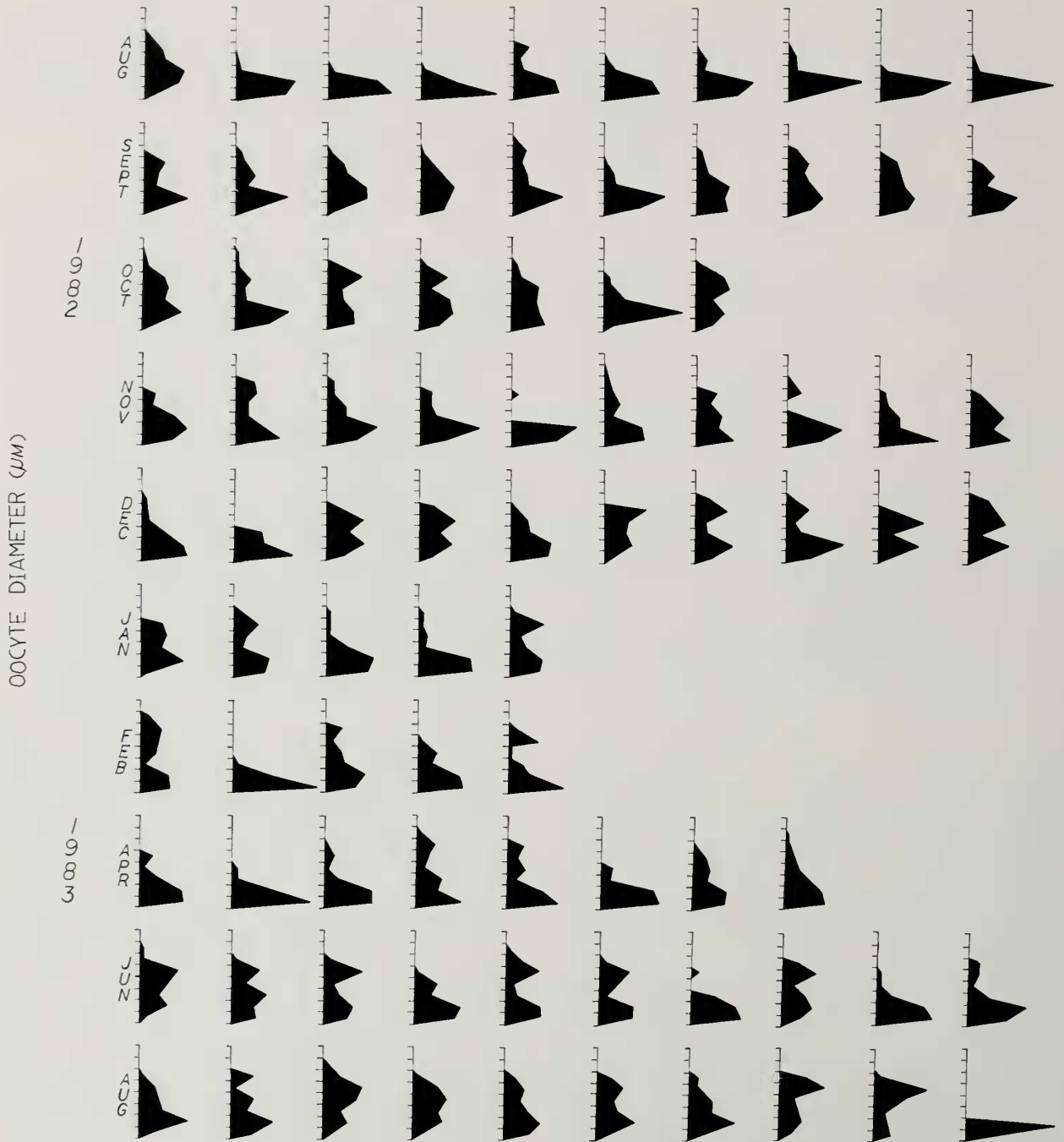


Figure 1

Solemya reidi. Monthly size-class distributions of oocytes in individuals from August 1982 to August 1983. Significant within monthly variation in distribution exists in all months (heterogeneity G-test, $P < 0.001$). Variation in distributions between months is also significant (heterogeneity G-test, $P < 0.001$). Oocyte diameter increments and percent distribution as in Figure 2.

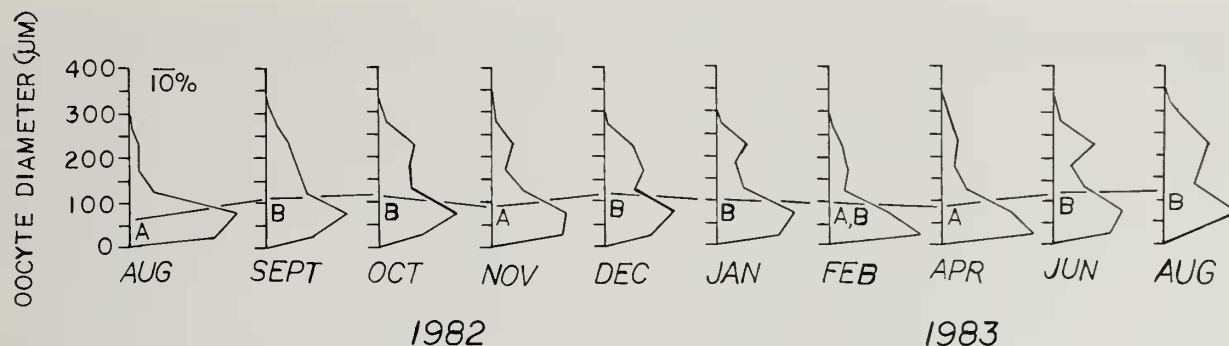


Figure 2

Solemya reidi. Size-class distributions of oocytes from August 1982 to August 1983, with individuals pooled within each month. Variations in distributions between months is significant (heterogeneity G-test, $P < 0.001$). Horizontal line connects mean monthly oocyte diameter values. Mean values labelled with different letters (a or b) are significantly different (Newman-Keuls multiple-range test, $P < 0.05$).

acquisition. All specimens of at least 25 mm in total shell length were sexually mature. Only individuals equal to or greater than this size were utilized.

Histological Procedures

Five to ten specimens of *Solemya reidi* of each sex from each collection were processed for histological analysis. Tissue pieces, containing gonad, were fixed in Bouin's fluid, dehydrated, embedded in paraffin wax (56–58°C), and sectioned at 7–10 μm . Slides were stained with standard eosin-hematoxylin (HUMASON, 1972). Oocyte size-frequency distributions were compiled for 5–10 females from each collection. The first 50 oocytes encountered that displayed nuclei were measured with the aid of an ocular micrometer. Mean oocyte diameters were calculated as the average of measurements along the longest and shortest axes of each oocyte. Statistical methods were used to test the significance of observed variations of oocyte size frequency between individuals within each sample, as well as between pooled monthly oocyte size frequencies (ZAR, 1974; SOKAL & ROHLF, 1981; GRANT & TYLER, 1983b).

RESULTS

Size-frequency distributions of oocyte diameters for individual female *Solemya reidi* are presented in Figure 1. The same data with individuals pooled within months are shown in Figure 2. Significant differences in size-class distributions of oocytes were observed between individuals in all months (heterogeneity G-test, $P < 0.001$) and among months ($P < 0.001$) (SOKAL & ROHLF, 1981). The presence of two separate, concurrent populations of oocytes in each individual is indicated by double peaks in many of the individual size-frequency distributions (Figure 1).

An analysis of variance showed no obvious trend in mean oocyte diameter over the period of collection (Figure 2). Mean oocyte diameters in August 1982, November

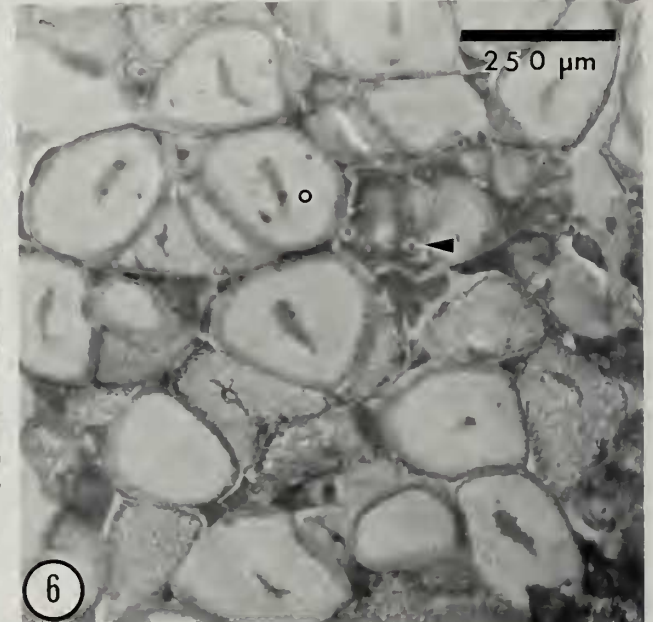
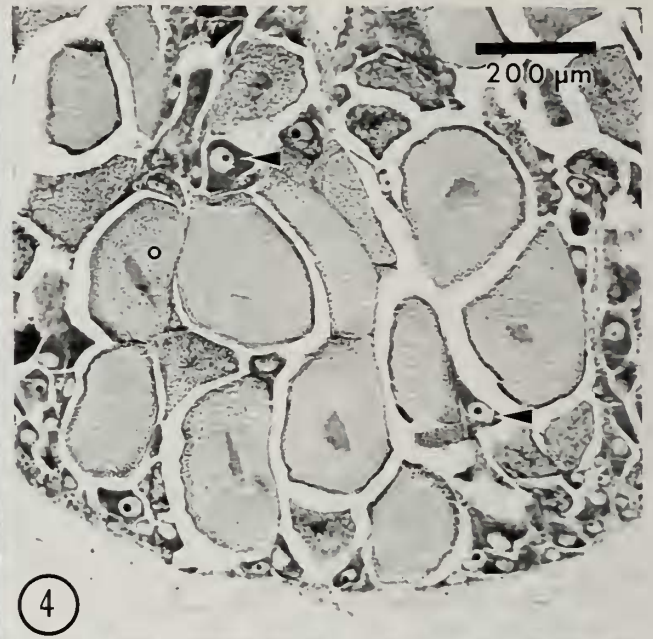
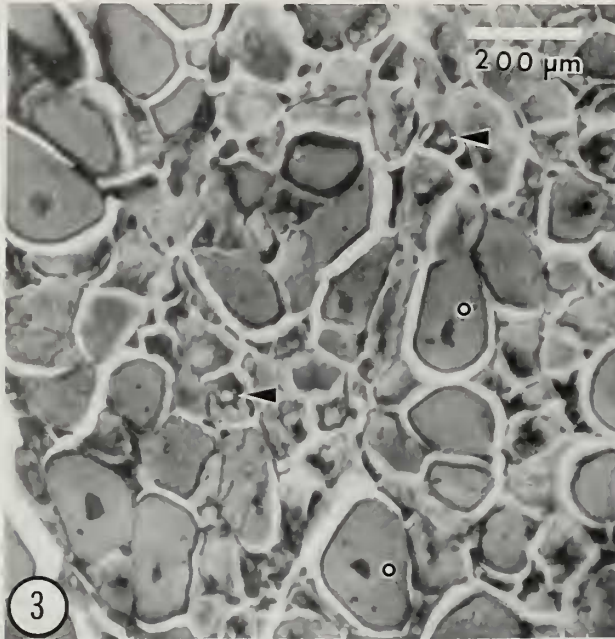
1982, and April 1983 were significantly different from those in September 1982, October 1982, December 1982, January 1983, June 1983, and August 1983 (Newman-Keuls multiple range test, $P < 0.05$).

The gonad of *Solemya reidi* is located deep within the base of the foot and extends alongside the posterior margin of the anterior adductor muscle. It was deemed inappropriate to construct stages of ovary development based on qualitative characters, as used previously in many studies of bivalve reproduction (SASTRY, 1979), owing to the wide range of oocyte diameters encountered in individuals, ranging from 24 to over 300 μm , and to the presence of two distinct populations of oocytes in each individual female (Figures 3–6). In rare cases, partially spent females were encountered (Figure 5). Even in these cases two populations of oocytes were apparent.

Histology of testes resembled that in other bivalves (SASTRY, 1979). No qualitative classification of the reproductive state of the testes was attempted, because the majority of the individuals were identical in histological appearance, with spermatogonia and mature spermatozoa present in almost all specimens (Figures 7, 8). In rare cases, testes were noted that were partially spent (Figures 9, 10). Ovaries were orange in color when ripe and full of eggs, but were black when less than ripe. Testes ranged from olive-green to white in color.

DISCUSSION

Several lines of evidence, including yearly data on the oocyte size frequencies, indicate that reproduction of *Solemya reidi* in Alberni Inlet, B.C., is continuous. Spawning of laboratory held *S. reidi* was observed in all seasons of the year (GUSTAFSON & REID, 1986). A "resting" or "inactive" phase of the gonads, common in many seasonally reproducing bivalves, was not observed. Mature spermatozoa were present in virtually all males throughout the



Explanation of Figures 3 to 6

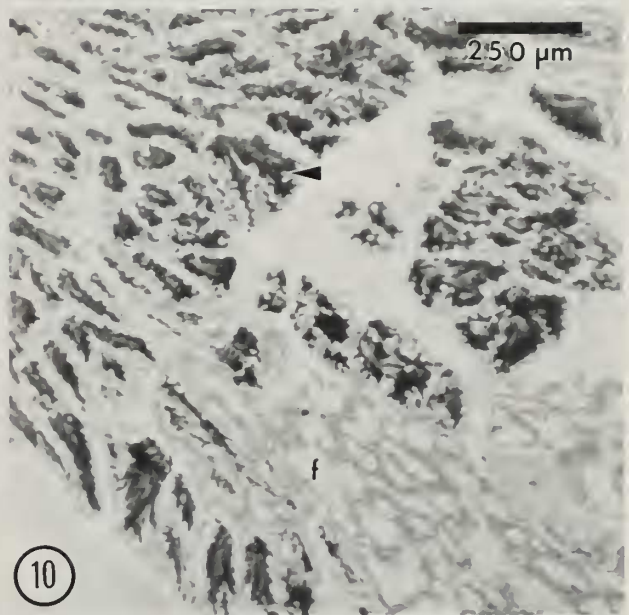
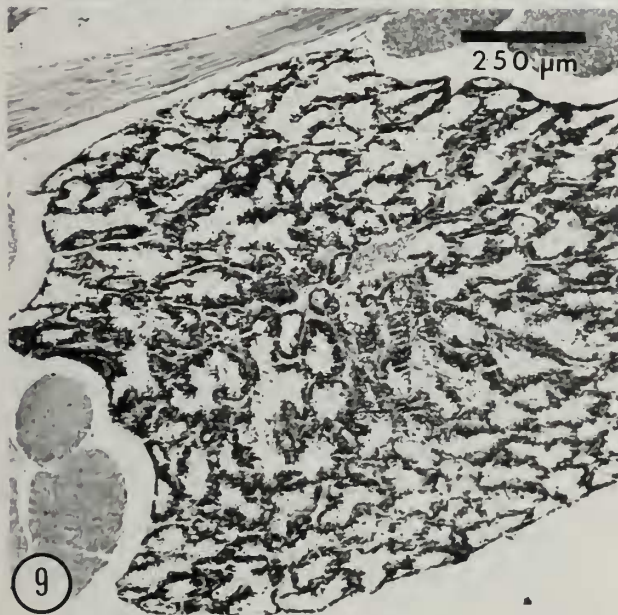
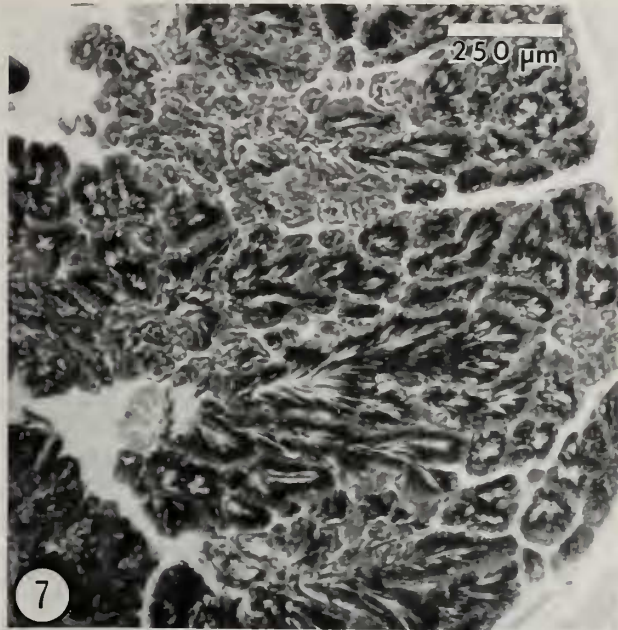
Figure 3. *Solemya reidi*. Photomicrograph of ovary showing development of two populations of oocytes; one large, over 150 μm in diameter (o) and one smaller, less than 50 μm (arrowheads).

Figure 4. *Solemya reidi*. Photomicrograph of ovary. Large ripe oocytes (o) fill the follicles, while small oocytes belonging to a second, developing population of oocytes, line the follicle walls (arrowheads).

Figure 5. *Solemya reidi*. Photomicrograph of partially spent ovary.

The large empty spaces in the follicles (f) indicate that spawning has recently occurred. A few remaining ripe oocytes (o) are spread throughout the ovary, while a second population of developing oocytes (arrowheads) line the follicle walls.

Figure 6. *Solemya reidi*. Photomicrograph of ripest ovary encountered. Large, ripe oocytes (o) are numerous; arrowhead points to developing oocytes of the next oocyte generation.



Explanation of Figures 7 to 10

Figure 7. *Solemya reidi*. Photomicrograph of testes showing follicles filled with mature spermatozoa.

Figure 8. *Solemya reidi*. Photomicrograph of ripe testes showing detail of follicles full of mature, tailed spermatozoa.

Figure 9. *Solemya reidi*. Photomicrograph of partially spent testes. Centers of follicles are depleted of spermatozoa.

Figure 10. *Solemya reidi*. Photomicrograph of partially spent testes showing some follicles (f) full of mature spermatozoa (arrowheads) and others totally depleted of germ cells.

year, except in those rare cases where partially spent individuals were noted.

Ovary histology (Figures 3-6) and oocyte size-frequency distributions (Figure 11) indicate that two readily distinguishable oocyte populations are present in each fe-

male, with the smaller oocytes being located along the follicle walls. Development of each population of oocytes within individuals is therefore synchronous, with spawning of the larger population of oocytes being more or less complete in each individual. Oogenesis is, however, asyn-

chronous within the population as illustrated by bimodal peaks of oocyte size-frequency distributions in each month (Figure 1) and the lack of a significant seasonal pattern in mean oocyte diameters or in pooled monthly oocyte size-frequency distributions (Figure 2). The concurrent development of two populations of oocytes in individual *Solemya reidi* indicates an ability to begin production of a future batch of oocytes before the current population is fully ripe.

Reproductive cycles may occur in populations of bivalves on an annual, semiannual, or continual basis. Continuous reproduction in a bivalve population can follow one of two patterns. A portion of the population can either be in a ripe reproductive state throughout the year, as in *Nucula cancellata* Jeffreys, 1881 (SCHELTEMA, 1972) and *Solemya reidi* (this study), or each individual can display all stages of gametogenesis simultaneously, as in *Nuculana pontonia* (Dall), *Nucula darella* Dall, and *Bathycarca* sp. (ROKOP, 1979). In the first case, each individual is capable of spawning out completely, whereas in the latter case individual gametogenesis is non-cyclic and year-round reproduction occurs both at the population and the individual levels.

In the tropics, where the temperature remains above the critical levels for spawning and food supplies are non-cyclic, many bivalves reproduce continuously (SASTRY, 1979; BRALEY, 1982; WALTER, 1982; LOPEZ & GOMEZ, 1982a, b). Likewise, in the deep sea where similar conditions prevail, many bivalves, particularly deposit-feeding protobranchs, spawn continuously (SCHELTEMA, 1972; ROKOP, 1974, 1979). Some degree of continuous breeding has also been reported for the temperate-zone and continental-shelf bivalves *Thyasira gouldi* (Philippi) (BLACKNELL & ANSELL, 1975), *Modiolus modiolus* (Linnaeus) (BROWN, 1984), *Abra nitida* (Müller) (BROWN, 1982) and *Lucinoma borealis* (Linnaeus) (TUNBERG, 1984).

Although *Solemya reidi* breeds continuously, some deep-sea (LIGHTFOOT *et al.*, 1979) and most continental-shelf protobranchs (LEBOUR, 1938; OCKELMANN, 1958; ANSELL, 1974; ANSELL & PARULEKAR, 1978; DAVIS & WILSON, 1983) breed on an annual basis. On the other hand, *Nucula nitidosa* Winckworth (= *N. nitida* Sowerby) from the German Bight are reproductively active from September to April and, in addition, young *N. nitidosa* can be found in all seasons (RACHOR, 1976).

In contrast to *Solemya reidi*, the shallow-water species *S. velum* Say reportedly spawns seasonally in late spring to mid-summer on the northeast coast of the U.S.A. (J. Pechenik, personal communication), whereas larvae of this species were found to settle only in winter and early spring in Bogue Sound, North Carolina, U.S.A. (WATZIN, 1986).

In the past, there has been considerable confusion as to the location of the gonad in *Solemya* spp. PELSENEER (1891) and STEPELL (1899) stated that the gonad of *S. togata* Poli occupies the interior of the basal part of the foot, whereas YONGE (1939:95) figures the gonad as dorsal to

the gills and hypobranchial gland in the same species. Furthermore, MORSE (1913:269) incorrectly identifies the gonad as "hepatic follicles" in *S. velum*. Descriptions of the habits and nutritive capabilities of *S. parkinsoni* Smith (OWEN, 1961) and *S. reidi* (REID, 1980) agree that the gonad occupies a large part of the interior of the foot and in the latter species extends into the tissue bordering the anterior adductor.

Much of the confusion over the location of the gonad in *Solemya* spp. is likely due to the unusual coloration and consistency of the ovary when in an unripe condition. The developing ovary of *S. reidi* is often black with few or no visible oocytes, whereas the ripe ovary is orange. REID (1980) commented on the dark color of the "gonad" in *S. reidi*, which "had a resemblance to digestive diverticula" of other bivalves. The present study has determined that the gonad of *S. reidi* occupies the interior of the basal part of the foot and extends into the mantle tissue bordering the posterior aspect of the anterior adductor muscle.

In conclusion, no reliable evidence was found of synchronization of breeding activity between individuals in a population of *Solemya reidi* from Alberni Inlet, B.C., Canada. Consequently, spawning in this population most likely occurs on a continuous basis throughout the year.

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LITERATURE CITED

- ANSELL, A. D. 1974. Seasonal changes in the biochemical composition of the bivalve *Nucula sulcata* from the Clyde Sea area. *Mar. Biol.* 25:101-108.
- ANSELL, A. D. & A. H. PARULEKAR. 1978. On the rate of growth of *Nuculana minuta* (Müller) (Bivalvia; Nuculanidae). *Jour. Moll. Stud.* 44:71-82.
- BERNARD, F. R. 1980. A new *Solemya* s. str. from the north-eastern Pacific (Bivalvia: Cryptodonta). *Venus* 39:17-23.
- BLACKNELL, W. M. & A. D. ANSELL. 1975. Features of the reproductive cycle of an arctic bivalve from a Scottish sea loch. VIII Europ. Mar. Biol. Symp., Publ. Staz. Zool. Napoli 39(Suppl. 1):26-52.
- BRALEY, R. 1982. Reproductive periodicity in the indigenous oyster *Saccostrea cucullata* in Sasa Bay, Apra Harbor, Guam. *Mar. Biol.* 69:165-173.
- BROWN, R. A. 1982. Reproduction of *Abra nitida* (Müller) (Bivalvia) in the southern Skagerrak. *Sarsia* 67:55-60.
- BROWN, R. A. 1984. Geographical variations in the reproduc-

- tion of the horse mussel, *Modiolus modiolus* (Mollusca: Bivalvia). Jour. Mar. Biol. Assoc. U.K. 64:751-770.
- DAVIS, J. P. & J. G. WILSON. 1983. The population structure and ecology of *Nucula turgida* (Leckenby and Marshall) in Dublin Bay. Prog. Underwat. Sci. (N.S.) 8:53-60.
- FELBECK, H. 1983. Sulfide oxidation and carbon fixation by the gutless clam *Solemya reidi*: an animal-bacteria symbiosis. Jour. Comp. Physiol. 152B:3-11.
- FELBECK, H., J. J. CHILDRESS & G. N. SOMERO. 1983. Biochemical interactions between molluscs and their algal and bacterial symbionts. In: P. W. Hochachka (ed.), The Mollusca, 2:331-358. Academic Press: New York.
- FISHER, C. R. & J. J. CHILDRESS. 1984. Carbon fixation and translocation in *Solemya reidi* (Bivalvia: Protobranchia). Amer. Zool. 24:57A.
- GRANT, A. & P. A. TYLER. 1983a. The analysis of data in studies of invertebrate reproduction. I. Introduction and statistical analysis of gonad indices and maturity indices. Int. Jour. Invert. Reprod. 6:259-269.
- GRANT, A. & P. A. TYLER. 1983b. The analysis of data in studies of invertebrate reproduction. II. The analysis of oocyte size/frequency data, and comparison of different types of data. Int. Jour. Invert. Reprod. 6:271-283.
- GUSTAFSON, R. G. & R. G. B. REID. 1986. Development of the pericalymma larva of *Solemya reidi* Bernard 1980 (Bivalvia, Cryptodonta, Solemyidae) as revealed by light and electron microscopy. Mar. Biol. 93:411-427.
- HAND, S. C. & G. N. SOMERO. 1983. Energy metabolism pathways of hydrothermal vent animals: adaptations to a food-rich and sulfide-rich deep sea environment. Biol. Bull. 165:167-181.
- HUMASON, G. L. 1972. Animal tissue techniques. W. H. Freeman: San Francisco. 641 pp.
- LEBOUR, M. V. 1938. Notes on the breeding of some lamellibranchs from Plymouth, and their larvae. Jour. Mar. Biol. Assoc. U.K. 23:119-144.
- LIGHTFOOT, R. H., P. A. TYLER & J. D. GAGE. 1979. Seasonal reproduction in deep-sea bivalves and brittlestars. Deep-Sea Res. 26A:967-973.
- LOPEZ, M. D. G. & E. D. GOMEZ. 1982a. Reproductive cycles of the oysters *Crassostrea echinata* and *C. lugubrius* in Calatagan, Batangas, Philippines. Kalikasan, Phil. Jour. Biol. 11:57-73.
- LOPEZ, M. D. G. & E. D. GOMEZ. 1982b. Reproductive cycle of the brown mussel *Modiolus metcalfei* in Calatagan, Batangas. Kalikasan, Phil. Jour. Biol. 11:74-82.
- MCMAHON, R. F. & R. G. B. REID. 1984. Respiratory responses of the gutless bivalve, *Solemya reidi*, to temperature, hypoxia, HS⁻, and dissolved organic matter. Amer. Zool. 24:136A.
- MORSE, E. S. 1913. Observations on living *Solenomya*. Biol. Bull. 25:261-281.
- OCKELMANN, K. W. 1958. The zoology of East Greenland Lamellibranchiata. Meddr. Gronl. 122:1-256.
- OWEN, G. 1961. A note on the habits and nutrition of *Solemya parkinsoni* (Protobranchia: Bivalvia). Quart. Jour. Microsc. Sci. 102:15-21.
- PELSENEER, P. 1891. Contribution à l'étude des lamellibranchés. Arch. Biol., Paris 11:147-312.
- POWELL, M. A. & G. N. SOMERO. 1985. Sulfide oxidation occurs in the animal tissue of the gutless clam, *Solemya reidi*. Biol. Bull. 169:164-181.
- POWELL, M. A. & G. N. SOMERO. 1986. Hydrogen sulfide oxidation is coupled to oxidative phosphorylation in mitochondria of *Solemya reidi*. Science 233:563-566.
- RACHOR, E. 1976. Structure, dynamics and productivity of a population of *Nucula nitidosa* (Bivalvia: Protobranchia). Ber. Dt. Wiss. Kommn. Meeresforsch. 24:296-331.
- REID, R. G. B. 1980. Aspects of the biology of a gutless species of *Solemya* (Bivalvia: Protobranchia). Can. Jour. Zool. 58:386-393.
- REID, R. G. B. & F. R. BERNARD. 1980. Gutless bivalves. Science 208:609-610.
- ROKOP, F. J. 1974. Reproductive patterns in the deep-sea benthos. Science 186:743-745.
- ROKOP, F. J. 1979. Year-round reproduction in the deep-sea bivalve molluscs. Pp. 189-198. In: S. E. Stancyk (ed.), Reproductive ecology of marine invertebrates. Univ. S. Carolina Press: Columbia.
- SASTRY, A. N. 1979. Pelecypoda (excluding Ostreidae). In: A. C. Giese & J. S. Pearse (eds.), Reproduction of marine invertebrates, 5:113-292. Academic Press: New York.
- SHELTEMA, R. S. 1972. Reproduction and dispersal of bottom dwelling deep-sea invertebrates: a speculative summary. Pp. 58-66. In: R. W. Brauer (ed.), Barobiology and the experimental biology of the deep-sea. Univ. N. Carolina Press: Chapel Hill.
- SOKAL, R. R. & F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman: San Francisco. 859 pp.
- STEMPELL, W. 1899. Zur Anatomie von *Solemya togata* Poli. Zool. Jb. Abt. Anat. Ont. Tiere 13:89-170.
- TUNBERG, B. 1984. Aspects of the population ecology of *Lucinoma borealis* (L.) (Bivalvia) in Raunefjorden, western Norway. Jour. Exp. Mar. Biol. Ecol. 81:87-106.
- WALTER, C. 1982. Reproduction and growth in the tropical mussel *Perna viridis* (Bivalvia, Mytilidae). Kalikasan, Phil. Jour. Biol. 11:83-97.
- WATZIN, M. C. 1986. Larval settlement into marine soft-sediment systems: interactions with the meiofauna. Jour. Exp. Mar. Biol. Ecol. 98:65-113.
- YONGE, C. M. 1939. The protobranchiate Mollusca: a functional interpretation of their structure and evolution. Phil. Trans. Roy. Soc. Lond., Ser. B, 230:79-147.
- ZAR, J. H. 1974. Biostatistical analysis. Prentice-Hall: Englewood Cliffs, New Jersey. 620 pp.